REMARKS

A check in the amount of $475 for the fee for a three-month extension of time is included with this response. Any fee that may be due in connection with this application, including a fee for an extension of time, may be charged to Deposit Account No. 06-1050. If a Petition for extension of time is needed, this paper is to be considered such Petition. A change of address for the undersigned accompanies this response.

Claims 1-55, 58-60, 63-76, 86, 88-124 and 127-144 are pending in this application. Claim 125 is cancelled herein without prejudice or disclaimer. Claims 2, 88 and 100 are amended herein in order to correct minor grammatical errors. Claim 124 is amended herein to more distinctly claim the subject matter and to incorporate the limitations of claim 125, which is cancelled herein. Claim 124 is further amended to recite that the mass-modifying functionality increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Basis for the amendment is found throughout the specification (for example, see page 19, lines 7-10; page 28, lines 27-29; and page 81, lines 5-7). No new matter is added.

Basis for added claim 129 can be found throughout the specification (for example, see page 28, lines 2-4). Basis for added claim 130 can be found throughout the specification (for example, see page 28, lines 9-15). Basis for added claim 131 can be found throughout the specification (for example, see page 28, lines 16-25). Basis for added claim 132 can be found throughout the specification (for example, see page 30, lines 8-11). Basis for added claims 133-135 can be found throughout the specification (for example, see page 25, lines 7-20). Basis for added claim 13 can be found throughout the specification (for example, see page 30, line 30 through page 31, line 3). Basis for added claims 137 and 138 can be found throughout the specification (for example, see page 31, lines 17-29). Basis for added claims 139 and 140 can be found throughout the specification (for example, see page 32, lines 10-14). Basis for added claim 141 can be found throughout the specification (for example, see page 31, line 27

through page 32, line 3). Basis for added claim 142 can be found throughout the specification (for example, see page 32, lines 5-9). Basis for added claim 143 can be found throughout the specification (for example, see page 11, lines 23-25). Basis for added claim 144 can be found throughout the specification (for example, see page 15, lines 14-15). No new matter is added.

THE REJECTION OF CLAIMS 1-27, 29-55, 58-60, 63-70, 73-76, 86, 88-125, 127 AND 128 UNDER 35 U.S.C. §103(a)

Claims 1-27, 29-55, 58-60, 63-70, 73-76, 86, 88-125, 127 and 128 are rejected under 35 USC § 103(a) over Köster (U.S. 5,605,798) in view of Cantor (U.S. 5,503,980), because Köster allegedly teaches all elements of the claimed subject matter except hybridizing each member of a set of nucleic acid fragments to a target array of probes and identifying hybridized probes by determining molecular weights of nucleic acids in the target array, but Cantor allegedly cures this defect. This rejection is respectfully traversed.

RELEVANT LAW

Under 35 U.S.C. §103, in order to set forth a case of *prima facie* obviousness, the differences between the teachings in the cited reference must be evaluated in terms of the whole invention, and the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. *See, e.g., Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 1462, 221 U.S.P.Q.2d 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); *see, also, In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963).

In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

Further, that which is within the capabilities of one of ordinary skill in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.*, 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

THE CLAIMS

Claim 1 is directed to a method for sequencing a target nucleic acid, which includes the steps of providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid; hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, wherein each probe includes a single-stranded portion including a variable region such that each member of the set hybridizes to a member of the array of probes; and determining molecular weights of nucleic acids in the target array to identify hybridized probes, and determining the sequence of the target nucleic acid based upon the hybridized probes. Claims 2-55, 58-60, 63-76, 88-123 and 128 depend from claim 1 and are directed to various embodiments thereof.

Claim 124 is directed to an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion; each single-stranded portion includes a variable sequence; the array of probes has sufficient sequence diversity in the variable regions to hybridize to all of a target

nucleic acid molecule with complete or nearly complete discrimination; the array is attached to a solid support including a matrix material that facilitates the volatilization of nucleic acids for mass spectrometry; and the array includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Claims 129-144 depend from claim 124 and are directed to various embodiments thereof. Claim 127 is directed to a system that includes a mass spectrometer, a computer and the array of claim 124.

TEACHINGS OF THE CITED ART

Köster (U.S. Patent 5,605,798)

Köster teaches methods of detecting nucleic acids using mass spectrometry to determine a molecular weight. Köster is not directed to methods of sequencing. Köster does not teach or suggest any methods for sequencing a target nucleic acid molecule. Köster does not teach or suggest any methods that involve sequencing by hybridization in which a set of target molecules is hybridized to a set of probes, nor such a method in which hybrids are detected by determining their molecular weight so that the sequence of the target can be constructed by identifying the hybridized probes.

Köster does not teach or suggest an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion and the single-stranded portion includes a variable region. Köster does not teach or suggest an array that includes a nucleic acid probe having at least one mass-modifying functionality that introduces a distinction detectable by mass spectrometry that increases the discrimination between at least two nucleic acids.

Köster does not teach or suggest a method such that each member of a set of nucleic acid fragments hybridizes to a member of an array of probes and the molecular weights of the nucleic acids in the target array are determined to identify hybridized probes, whereby the sequence of a target nucleic acid molecule is determined.

Cantor (U.S. Patent 5,503,980)

Cantor teaches positional sequencing by hybridization. Cantor teaches probes having a double-stranded portion, a single-stranded portion, and a random sequence within the single-stranded portion that is determinable (col. 5, lines 40-45). In one embodiment, Cantor teaches a method for determining a nucleotide sequence by positional hybridization (col. 7, lines 63 through col. 8, line 6). Cantor teaches using hybridization chips with large probe arrays subsequently hybridized with target nucleic acid and determining the target nucleotide sequence by analysis of the hybridization pattern on the chip, which provides a fingerprint identification of the target nucleotide sequence (col. 7, lines 6-10).

Cantor does not teach or suggest a method such that each member of a set of nucleic acid fragments hybridizes to a member of an array of probes and the molecular weights of the nucleic acids in the target array are determined to identify hybridized probes, whereby the sequence of the target nucleic acid is determined. Cantor does not teach an array that is attached to a solid support that includes a matrix material that facilitates the volatilization of nucleic acids for mass spectrometry. Cantor does not teach or suggest an array that includes a nucleic acid probe having at least one mass-modifying functionality that introduces a distinction detectable by mass spectrometry that increases the discrimination between at least two nucleic acids. Cantor does not teach a system that includes a mass spectrometer, a computer and the array of nucleic acid probes as instantly claimed.

ANALYSIS

Claims 1-27, 29-55, 58-60, 63-70, 73-76, 86, 88-125, 127 and 128 have been rejected as a "group" in the Office Action. As set forth above and discussed in detail below, the pending claims are directed to (1) methods of sequencing a target nucleic acid [claims 1-55, 58-60, 63-76, 88-123 and 128]; (2) an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion; each single-stranded portion includes a variable sequence; the array of probes has sufficient sequence diversity in the variable

regions to hybridize all of a target sequence with complete or nearly complete discrimination; the array is attached to a solid support including a matrix material that facilitates the volatilization of nucleic acids for mass spectrometry; and the array includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry [claim 124]; and (3) a system that includes a mass spectrometer, a computer and the array of claim 124 [claims 86 and 127]. Accordingly, the traversal of the rejection is discussed below with reference to each of the particular methods, arrays and systems claimed in the instant claims.

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for each of the groups of claims for the following reasons.

1. CLAIMS 1-55, 58-60, 63-76 AND 88-123

(1) There would have been no motivation to have combined the teachings of Köster with those of Cantor

The Method of Köster is Not Complementary to the Method of Cantor

There is no motivation to have combined the teachings of Köster and Cantor because each reference describes methods that are not complementary to the other. Köster discloses a variety of different embodiments for detecting a target nucleic acid molecule in a sample. For example, Köster, in one embodiment, discloses attaching a capture sequence chosen to specifically hybridize with a complementary sequence of the target nucleic acid to a solid support, whereby the target is displayed (FIG. 1A and col. 4, lines 59-67). The target nucleic acid molecule includes a target detection site, and the presence of the target nucleic acid is determined by hybridizing a detector nucleic acid sequence to the target detection site and detecting the detector by mass spectrometry. The method does not result in the sequence of a target nucleic acid, but rather determines whether a particular target is present in a nucleic acid by virtue of hybridization of a detector oligonucleotide to targets displayed on a

solid support and detecting the detector oligonucleotide by mass spectrometry.

Köster teaches at col. 4, lines 39-49 that

> The processes of the invention provide for increased accuracy and reliability of nucleic acid detection by mass spectrometry. In addition, the processes allow for rigorous controls to prevent false negative or positive results. The processes of the invention avoids electrophoretic steps; labeling and subsequent **detection of a label**. In fact it is estimated that the entire procedure, including nucleic acid isolation, amplification, and mass spec analysis requires only about 2-3 hours time. Therefore the instant disclosed processes of the invention are faster and less expensive to perform than existing DNA detection systems.

Köster also discloses an array of detector oligonucleotides, but does not suggest any methods in which such array is used for sequencing. Köster teaches the direct detection of nucleic acid molecules by mass spectrometry.

Cantor teaches, in one embodiment, a method for determining a nucleotide **sequence** by positional hybridization. Cantor teaches, at col. 10, lines 48-55, that

> Hybridization of target nucleic acids could be determined by adding a detectable label, such as a labeled antibody, which will specifically recognize only hybridized targets or, alternatively, unhybridized target is washed off and labeled target specific antibodies are added. In either case, appearance of label on the solid support indicates the presence of nucleic acid target hybridized to the probe and consequently, within the biological sample.

Cantor further teaches at col. 7, lines 32-39 that

> Label may be directly or indirectly detected using scintillation fluid or a PhosphorImager, chromatic or fluorescent labeling, or mass spectrometry. Other, more advanced methods of detection include evanescent wave detection of surface plasmon resonance of thin metal film labels such as gold, by, for example, the BIAcore sensor sold by Pharmacia, or other suitable biosensors.

See also col. 9, lines 55-60, which teaches

> A further embodiment of the present invention is a method wherein the target nucleic acid has a first detectable label at a terminal site and a second detectable label at an internal site. It is also preferred that the first and second detectable labels are chromatic or fluorescent chemicals or molecules which are detectable by mass spectrometry.

The only use of mass spectrometry taught by Cantor is using mass spectrometry to detect labels. Thus, Cantor teaches using a detectable label to determine the hybridization of target nucleic acids to probes. Cantor does not teach or suggest using detecting hybridization to a probe based on a molecular weight, such as is determined by mass spectrometry of a nucleic acid molecule to detect a target nucleic acid.

Hence, Cantor teaches using mass spectrometry to detect labels, while Köster teaches away from using labels to detect a nucleic acid. Köster teaches that its processes provide increased accuracy and reliability of nucleic acid detection by mass spectrometry **by avoiding subsequent detection of a label.** Hence, Köster teaches away from using a label to detect a nucleic acid molecule. Thus, the method taught by Köster is not complementary to the method taught by Cantor. Therefore, there is no motivation to combine the teachings of Köster with Cantor. Further, the proposed combination of the prior art would change the principle of operation of the Köster processes, since Cantor teaches detecting a nucleic acid using a label, while Köster teaches direct detection of the nucleic acid by mass spectrometry, thus obviating the need for a label. Köster teaches that by eliminating the need for detecting a label, its procedure, including nucleic acid isolation, amplification, and mass spec analysis requires only about 2-3 hours time. Hence, the Köster method of detection of nucleic acids and the Cantor method of sequencing nucleic acids were complete methods unto themselves and were mutually exclusive. There would have been no motivation to have combined the Köster method of detection of nucleic acids and the Cantor method of sequencing nucleic acids.

Sequencing and Nucleic Acid Detection are very Different Methods

Moreover, Köster and Cantor are directed to completely different methods. Köster is directed to methods of detecting nucleic acids in a sample; Cantor is directed to methods of sequencing a target nucleic acid molecule. Methods of sequencing and methods of detecting are very different methods. In general, in methods for detecting a nucleic acid, for each nucleic acid to be detected, the

molecular mass of only a single nucleic acid molecule is measured, i.e., the molecular mass of the actual nucleic acid to be detected or of a detector oligonucleotide is detected. There are no nested sets of fragments generated. Thus, there are no molecular mass reference points of related subset fragments for comparison in methods of detection. Therefore, for detection methods, it is critical that the mass determination of each individual nucleic acid to be detected be as accurate as possible. Accuracy is at a premium in detection methods because a rapid detection is of little value if it is not highly accurate in every measurement.

In contrast, in methods of determining a sequence by the method of Cantor, the identity, and in some embodiments, the sequence, of a plurality of probes are determined. The sequence is inferred based upon the probes to which the target binds. Hence the method requires reaction with a plurality of different probes; whereas detection requires a reaction with a single probe. There is no relationship between the methods. Thus, the methods of Köster (which are directed to detection of nucleic acid molecules by mass spectrometry) are directed at a very different process than the methods of Cantor (which are directed to methods of sequencing a nucleic acid molecule by sequencing by hybridization). The objectives to achieve and that are achieved by Köster differ from those of Cantor. Therefore, one of ordinary skill in the art would not have been motivated to combine the method of detecting taught by Köster with the method of sequencing taught by Cantor.

Notwithstanding the lack of motivation to have combined the teaching of the references, the combination does not result in the instantly claimed methods.

(2) Notwithstanding the lack of motivation, the combination of the teachings of Köster with the teachings of Cantor does not result in the instantly claimed methods.

Köster does not teach or suggest a sequencing method that includes determining molecular weights of nucleic acids in a target array (*i.e.*, a set of nucleic acid fragments from a single target nucleic acid hybridized to an array of

nucleic acid probes having a structure that includes a double-stranded portion and a single-stranded portion with a variable sequence within the single-stranded region) to identify hybridized probes, and based upon the hybridized probes, determining the sequence of the target nucleic acid.

In fact, the Examiner states on page 8 of paper 1103 (the Office Action mailed December 19, 2003) that "Köster does not teach the method such that each member of the set hybridizes to a member of the array of probes and *determining molecular weights of nucleic acids in the target array to identify hybridized probes*" (emphasis added).

The teachings of Cantor *et al.* do not cure this deficiency. Cantor does not teach or suggest determining the molecular weights of nucleic acid fragments hybridized in a target array in order to identify hybridized probes and thereby determine the sequence of the target nucleic acid. The Examiner alleges that the Abstract, Example 2 and Example 4 of Cantor teaches a method that includes determining the molecular weights of nucleic acids in the array to identify targeted probes. Applicant respectfully disagrees. None of the sections cited by the Examiner, nor Cantor as a whole, provide the support suggested by the Examiner. For example, the Abstract recites:

> This invention is directed to methods for determining a nucleotide sequence of a nucleic acid using positional sequencing by hybridization, and to the creation of nucleic acids probes which may be used with these methods. This invention is also directed to diagnostic aids for analyzing the nucleic acid composition and content of biological samples, including samples derived from medical and agricultural sources.

Molecular weight is not mentioned in the Abstract. There is no teaching or suggestion in the Abstract of Cantor to determine the molecular weights of nucleic acid fragments hybridized in the target array in order to identify hybridized probes and thereby determine the sequence of the target nucleic acid.

Example 2 of Cantor does not teach or suggest an array of probes as instantly claimed. Example 2 of Cantor recites:

Preparation of model arrays.

Following the scheme shown in FIG. 2, in a single synthesis, all 1024 possible single-stranded probes with a constant 18 base stalk followed by a variable 5 base extension can be created. The 18 base extension is designed to contain two restriction enzyme cutting sites. Hga I generates a 5 base, 5' overhang consisting of the variable bases N_5. Not I generates a 4 base, 5' overhang at the constant end of the oligonucleotide. The synthetic 23-mer mixture will be hybridized with a complementary 18-mer to form a duplex which can then be enzymatically extended to form all 1024, 23-mer duplexes. These can be cloned by, for example, blunt end ligation, into a plasmid which lacks Not I sites. Colonies containing the cloned 23-base insert can be selected. Each should be a clone of one unique sequence. DNA minipreps can be cut at the constant end of the stalk, filled in with biotinylated pyrimidines, then cut at the variable end of the stalk, to generate the 5 base 5' overhang. The resulting nucleic acid can be fractionated by Qiagen columns (nucleic acid purification columns) to discard the high molecular weight material, and the nucleic acid probe will then be attached to a streptavidin-coated surface. This procedure could easily be automated in a Beckman Biomec or equivalent chemical robot to produce many identical arrays of probes.

The initial array contains about a thousand probes. The particular sequence at any location in the array will not be known. However, the array can be used for statistical evaluation of the signal to noise ratio and the sequence discrimination for different target molecules under different hybridization conditions. Hybridization with known nucleic acid sequences allows for the identification of particular elements of the array. A sufficient set of hybridizations would train the array for any subsequent sequencing task. Arrays are partially characterized until they have the desired properties. For example, the length of the oligonucleotide duplex, the mode of its attachment to a surface, and the hybridization conditions used, can all be varied, using the initial set of cloned DNA probes. Once the sort of array that works best is determined, a complete and fully characterized array can then be constructed by ordinary chemical synthesis.

The only mention of molecular weight in Example 2 is the fractionation of sample on a Qiagen column to discard the high molecular weight material (col. 13, lines 2-6). There is no teaching or suggestion in Example 2 of Cantor to determine the molecular weights of nucleic acid fragments hybridized in the target array in order

to identify hybridized probes and thereby determine the sequence of the target nucleic acid.

Example 4 of Cantor also does not teach or suggest an array of probes as instantly claimed. Example 4 of Cantor recites:

> **Extension of hybridized probe arrays with DNA polymerase.**
>
> Ligation ensures the fidelity of detection of the 3' terminal base of the target DNA. To ensure similar fidelity of detection at the 5' end of the duplex formed between the probe and the target, the probe-target duplex can be extended after ligation by one nucleotide using, for example, a labeled ddNTP (FIG. 4). This has two major advantages. First, specificity is increased because extension with the Klenow fragment of DNA polymerase requires a correctly base paired 3'-primer terminus. Second, using labeled ddNTPs one at a time, or a mixture of all four labeled with four different colors simultaneously, the identity of one additional nucleotide of the target nucleic acid can be determined as shown in FIG. 4. Thus, an array of only 1024 probes would actually have the sequencing power of an array of 4096 hexamers, in other words, a corresponding four-fold gain for any length used. In addition, polymerases work well in solid state sequencing methodologies quite analogous of the type proposed herein.

Molecular weight is not mentioned in Example 4. Example 4 of Cantor teaches using labels, such as differently colored labels, to determine the identity of any additional nucleotides added by the DNA polymerase (see col. 13, lines 63-67). There is no teaching or suggestion in Example 4 of Cantor to determine the molecular weights of nucleic acid fragments hybridized in the target array in order to identify hybridized probes and thereby determine the sequence of the target nucleic acid.

Other than the teaching in Example 2 directed to fractionation of a sample, the only other mention of molecular weight in Cantor is the Maxim and Gilbert sequencing technique, where terminally labeled DNA molecules are chemically cleaved at single base repetitions and then the molecular weight of each partially cleaved fragment is determined using electrophoresis to produce a pattern of fragments on a gel, whereby the DNA sequence can be read (col. 1, lines 24-35). Hence, none of the sections cited by the Examiner, nor Cantor as a whole,

teaches or suggests hybridizing a set of nucleic acid fragments, each containing a sequence that corresponds to a sequence of a target nucleic acid, to an array of nucleic acid probes to form a target array of nucleic acids, and determining molecular weights of nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined.

Combination of teachings of Köster and Cantor

Thus, even if Cantor teaches positional sequencing by hybridization that includes hybridizing a nucleic acid target that is at least partly single stranded to a set of nucleic acid probes, the combination of teaching of Köster and Cantor does not teach or suggest a method for sequencing a target nucleic acid that includes as a step determining the molecular weight of nucleic acid fragments hybridized in the target array in order to identify hybridized probes and thereby determine the sequence of the target nucleic acid. Hence, the combination of Köster and Cantor does not teach or suggest every element of claims 1-55, 58-60, 63-76 and 88-123 because neither reference, singly nor in combination, teaches or suggests a method that includes as a step determining the molecular weight of nucleic acid fragments hybridized in the target array in order to identify hybridized probes and thereby determine the sequence of the target nucleic acid. Thus, the combination of the teachings of Köster and Cantor does not result in the instantly claimed methods of claims 1-27, 29-55, 58-60, 63-70, 73-76 and 88-123.

2. CLAIMS 124 AND 125 and claims dependent thereon

Claims 124 and 125 are rejected under 35 USC § 103(a) over Köster (U.S. 5,605,798) in view of Cantor (U.S. 5,503,980). The Examiner alleges on pages 7 and 8 of Paper 1103 that:

> Köster teaches an array of nucleic acid probes, comprising a collection of probes, wherein each probe comprises a singe-stranded portion and a double-stranded portion (Figure 3);
>
> each single-stranded portion comprises a variable sequence (Figure 3 and Examples 1-2);
>
> the collection contains 4R probes, where R is the length of the variable region (Figures 1-3);

> the collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination (Example 1 and Claim 1 and Figure 1 and Column 4, lines 11-14 and Column 9, lines 28-43 and Figures 2-3); and
>
> the array is attached to a solid support comprising a matrix that facilitates volatilization of nucleic acids for molecular weight determination (Column 2, lines 14-33).

Applicant notes that the rejection appears to be set forth as a rejection under 35 USC § 103(a) over Köster (U.S. 5,605,798) in view of Cantor (U.S. 5,503,980), but fails to indicate what teachings of Cantor, if any, are combined with Köster. Furthermore, none of the sections cited by the Examiner, nor Köster as a whole, teach the instantly claimed arrays as suggested by the Examiner. Therefore, this rejection is respectfully traversed. It is respectfully submitted that the rejection as applied to claim 125 is moot in light of cancellation of claim 125 herein.

Köster does not teach or suggest the instantly claimed arrays.

Köster does not teach or suggest an array of nucleic acid probes where each probe includes a single-stranded portion and a constant double-stranded portion; each single-stranded portion includes a variable sequence; the array is attached to a solid support including a matrix material that facilitates the volatilization of nucleic acids for mass spectrometry; and the array includes a nucleic acid probe having at least one mass-modifying functionality that introduces a distinction detectable by mass spectrometry that increases the discrimination between at least two nucleic acids. None of the sections cited by the Examiner, nor Köster as a whole, provide the support suggested by the Examiner.

Köster does not disclose any arrays of probes that contain variable regions, such that the array of probes has sufficient sequence diversity in the variable regions to hybridize to all of a target nucleic acid molecule with complete or nearly complete discrimination. For instance, Example 1 is directed to an embodiment where a 50 nucleotide sequence (50-mer) attached to controlled pore glass beads serves as a template for separate hybridizations with a 26-mer ***or*** a 46-mer.

Köster at column 12, line 53. Oligonucleotide not bound to the polymer-bound template is removed by centrifugation and washing, and the beads are mixed with matrix and analyzed by MALDI-TOF mass spectrometry. If, arguendo, the 50-mer attached to the glass beads is construed to be a "probe" as used in the instant application, there is ***no*** variable region because the same 50-mer is attached to each of the controlled pore glass beads, and hence the sequence is identical. Alternatively, if the 26-mer or the 46-mer were considered to be the "probe," again there is no variable region, as the sequence of both the 26-mer and the 46-mer remains unvaried. Furthermore, Example 1 shows capture of a detector nucleic acid molecule on a solid support that is presenting a target molecule, and then detecting the hybridized detector by mass spectrometry. Applicant respectfully submits that the immobilized 50-mer does not have a double-stranded portion and a single-stranded portion, where the single-stranded portion includes a **variable** sequence. Example 1 of Köster does not teach or suggest that the array of probes has sufficient sequence diversity in the variable regions to hybridize all of a target sequence with complete or nearly complete discrimination. Example 1 does not teach or suggest a probe having at least one mass-modifying functionality that introduces a distinction detectable by mass spectrometry that increases the discrimination between at least two nucleic acids.

Example 2 of Köster does not teach or suggest the array of probes as instantly claimed. Example 2 recites:

> **Electrospray (ES) desorption and differentiation of an 18-mer and 19-mer**
>
> DNA fragments at a concentration of 50 pmole/μl in 2-propanol/10 mM ammonium carbonate (1/9, v/v) were analyzed simultaneously by an electrospray mass spectrometer.
>
> The successful desorption and differentiation of an 18-mer and 19-mer by electrospray mass spectrometry is shown in FIG. 11.

Example 2 of Köster does not teach or suggest an array of probes having a constant double-stranded portion and a single-stranded portion, where the single-stranded portion includes a **variable** sequence.

Figure 3 does not teach or suggest an array of probes having a **constant** double-stranded portion and a single-stranded portion, where the single-stranded portion includes a **variable** sequence. If the combination of the capture sequence and the target capture site in the array of FIG. 3 is construed to be the "double-stranded region" of the probes of the array, applicant respectfully submits that the double-stranded region is not constant. Every "probe" includes a different capture sequence C_x. FIGURE 3 of Köster does not teach or suggest that the array of probes has sufficient sequence diversity in the variable regions to hybridize all of a target sequence with complete or nearly complete discrimination.

Claim 1 of Köster also does not teach or suggest an array of nucleic acid probes as instantly claimed. Claim 1 of Köster recites:

> 1. A process for detecting a target nucleic acid sequence present in a biological sample, comprising the steps of:
> a) obtaining a nucleic acid molecule containing a target nucleic acid sequence from a biological sample;
> b) hybridizing a detector oligonucleotide with the target nucleic acid sequence, wherein at least one of the detector oligonucleotide or the target nucleic acid sequence has been conditioned;
> c) removing unhybridized detector oligonucleotide;
> d) ionizing and volatizing the product of step c); and
> e) detecting the detector oligonucleotide by mass spectrometry, wherein detection of the detector oligonucleotide indicates the presence of the target nucleic acid sequence in the biological sample.

Claim 1 of Köster is directed to a method that includes hybridizing a detector oligonucleotide with a target nucleic acid sequence. Claim 1 of Köster makes no mention of arrays of probes nor a mass-modified array. Claim 1 does not teach or suggest probes that include a single-stranded portion and a constant double-stranded portion where each single-stranded portion includes a variable sequence. Claim 1 of Köster does not teach or suggest that the array of probes has sufficient sequence diversity in the variable regions to hybridize all of a target sequence with complete or nearly complete discrimination. Claim 1 of Köster does not teach or suggest an array attached to a solid support including a matrix material that facilitates the volatilization of nucleic acids for mass spectrometry. Claim 1 of

Köster does not teach or suggest an array that includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acids. Hence, Claim 1 of Köster does not teach or suggest every element of the claimed subject matter.

FIGURE 1 also does not disclose an array of nucleic acid probes as instantly claimed. FIGURE 1 shows a process for performing mass spectrometry analysis on a target detection site (TDS) contained within a target nucleic acid molecule (T). A specific capture sequence (C) (FIGS. 1A and 1C) is attached to a solid support (SS) via a spacer (S). The capture sequence (C) hybridizes with a complementary sequence on the target nucleic acid molecule to immobilize the sequence to the solid support. In an alternative embodiment, a target nucleic acid containing a detection site (FIG. 1B) is immobilized to a spacer via a linkage L-L'. None of FIGS. 1A through 1C shows an "array of nucleic acid probes," but instead show only a single oligonucleotide attached to a solid support. Further, none of Figs. 1A-C show a probe containing a single-stranded portion having a variable region. None of FIGS. 1A-C of Köster teaches or suggests that the array of probes has sufficient sequence diversity in the variable regions to hybridize all of a target sequence with complete or nearly complete discrimination.

Köster teaches multiplexing at col. 4, lines 11-14 and col. 9, lines 28-43. For example, at col. 4, lines 11-14, Köster teaches that

> [i]n preferred embodiments, the target detection site is amplified prior to detection and the nucleic acid molecules are conditioned. In a further preferred embodiment, the target detection sequences are arranged in a format that allows multiple simultaneous detections (multiplexing).

At col. 9, lines 28-43, Köster teaches:

> For certain applications, it may be useful to simultaneously detect more than one (mutated) loci on a particular captured nucleic acid fragment (on one spot of an array) or it may be useful to perform parallel processing by using oligonucleotide or oligonucleotide mimetic arrays on various solid supports. "Multiplexing" can be achieved by several different methodologies. For example, several mutations can be simultaneously detected on one target sequence by

> employing corresponding detector molecules (e.g. oligonucleotides or oligonucleotide mimetics. However, the molecular weight differences between the detector oligonucleotides D1, D2 and D3 must be large enough so that simultaneous detection (multiplexing) is possible. This can be achieved either by the sequence itself (composition or length) or by the introduction of mass-modifying functionalities M1-M3 into the detector oligonucleotide. (FIG. 2)

FIGURES 2, 3 and 5, for example, show various methods of multiplexing (see col. 5, line 42 through col. 6, line 33). FIG. 2 is a diagram showing a process in which several mutations are simultaneously detected on one target sequence by employing a plurality of different detector oligonucleotides. FIG. 2 does not teach an array of probes, but instead shows only a single oligonucleotide attached to a solid support. FIG. 2 of Köster does not teach or suggest an array of probes having sufficient sequence diversity in the variable regions of the probes of the array to hybridize all of a target sequence with complete or nearly complete discrimination.

FIGS. 5 and 8 show multiplexing using an ordered array of different capture sequences. As discussed above, if the combination of the capture sequence and the target capture site is construed to be the double-stranded portion of the probe, neither FIG. 5 nor FIG. 8 teaches or suggests a probe that includes a constant double-stranded region, as each probe has a different capture sequence. Further, neither FIG. 5 nor FIG. 8 teaches or suggest a probe that includes a variable sequence in the single-stranded region. Neither FIG. 5 nor FIG. 8 of Köster teaches or suggests an array of probes having sufficient sequence diversity in the variable regions of the probes of the array to hybridize all of a target sequence with complete or nearly complete discrimination.

Thus, Köster does not teach or suggest an array of probes that include a single-stranded and a double-stranded region where the single-stranded region includes a *variable* sequence such that the array of probes has sufficient sequence diversity in the variable regions to hybridize to all of a target nucleic acid molecule with complete or nearly complete discrimination.

Thus, Köster does not teach or suggest an array of probes having sufficient sequence diversity in the variable regions of the probes of the array to hybridize all of a target sequence with complete or nearly complete discrimination. Thus, Köster does not teach or suggest every element of the claimed array.

Applicant notes that the rejection appears to be set forth as a rejection under 35 USC § 103(a) over Köster (U.S. 5,605,798) in view of Cantor (U.S. 5,503,980), but the Examiner fails to indicate what teachings of Cantor, if any, are combined with Köster. MPEP 2142 states:

> The initial burden is on the Examiner to provide some suggestion of the desirability of doing what the inventor has done. "To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or implied suggest the claimed invention or the Examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references. *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). See MPEP 2144-214.09 for examples of reasoning supporting obviousness rejections.

MPEP 706.02(j) states:

> After indicating that the rejection is under 35 USC § 103, the Examiner should set forth in the Office Action:
>
> (A) the relevant teachings of the prior art relied upon, preferably with reference to the relevant column or page number(s) and line number(s) where appropriate,
>
> (B) the difference or differences in the claim over the applied references,
>
> (C) the proposed modification of the applied reference(s) necessary to arrive at the claimed subject matter, and
>
> (D) an explanation why one of ordinary skill in the art at the time the invention was made would have been motivated to make the proposed modification.

Further, MPEP 706.02(j) recites:

> Where a reference is relied on to support a rejection, whether or not in a minor capacity, that reference should be positively included in the statement of the rejection. See *In re Hoch*, 428 F.2d 1341, 1342 n.3, 166 USPQ 406, 407 n.3 (CCPA 1970).

Applicant respectfully submits that the Examiner provides no indication that Cantor was relied upon in the rejection of claims 124 and 125. There is no reference to any specific teaching in Cantor or an explanation as to why one of ordinary skill in the art would have been motivated to make any modification of the arrays taught by Köster. The Examiner does not indicate where, if at all, Cantor cures the defects in Köster.

Notwithstanding this, the combination of teachings of Köster and Cantor does not result in the instantly claimed arrays. Cantor teaches arrays of probes that are partially double-stranded and partially single-stranded, where in all embodiments the target molecule is labeled for detection. Cantor does not teach or suggest arrays of probes in which the probes are labeled. Although Köster teaches mass-modification for discrimination among a plurality of nucleic acid molecules, Köster does not suggest modifying the arrays of Cantor such that the probes are mass modified. Therefore, the combination of the teachings of Köster with those of Cantor does not result in the instantly claimed arrays. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 86 and 127

Claims 86 and 127 are rejected because the examiner alleges that Köster teaches a system that includes a mass spectrometer, a computer and the array as claimed in claim 124, citing Col. 2, lines 33-45. As discussed above, Köster does not teach or suggest an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion; each single-stranded portion includes a variable sequence; the array of probes has sufficient sequence diversity in the variable regions to hybridize all of a target sequence with complete or nearly complete discrimination; the array is attached to a solid support including a matrix material that facilitates the volatilization of nucleic acids for mass spectrometry; and the array includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Hence,

Köster does not teach or suggest a system that includes a mass spectrometer, a computer and the array of claim 124.

As discussed above, there is no indication that Cantor was relied upon in the rejection of claims 124 and 125, and similarly, there is no indication that Cantor was relied upon in the rejection of claims 86 and 127. There is no reference to any specific teaching in Cantor or an explanation as to why one of ordinary skill in the art would have been motivated to make any modification of the arrays taught by Köster. The Examiner does not indicate where, if at all, Cantor cures the defects in Köster.

Thus, because Köster does not teach or suggest the array as claimed in claim 124, Köster does not teach or suggest the system as claimed in claims 86 and 127, which include the array of claim 124. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

THE REJECTION OF CLAIM 28 UNDER 35 U.S.C. §103(a)

Claim 28

Claim 28 is rejected under 35 U.S.C. §103(a) over Köster (U.S. 5,605,798) in view of Cantor (U.S. 5,503,980) and further in view of Weiss (U.S. 6,025,193) because the combination of Köster and Cantor allegedly teaches all elements of claim 28, except generation of thiol moieties by using Beucage reagent, but Weiss allegedly cures this defect. Applicant respectfully traverses the rejection.

THE CLAIM

Claim 28 ultimately depends from claim 1 and is directed to the embodiment thereof where the mass-modifying functionality is a thiol moiety The generated by using Beucage reagent.

RELEVANT LAW

See related section above.

TEACHINGS OF THE CITED ART

Köster (U.S. Patent 5,605,798)

See related section above.

Cantor (U.S. Patent 5,503,980)

See related section above.

Weiss (U.S. Patent 6,025,193)

Weiss teaches methods and compositions for diagnosing and treating pathological conditions related to a dopamine receptor abnormality, which includes administering a plasmid encoding an oligonucleotide anti-sense to one or more RNA molecules encoding one of the several dopamine receptors. The reference teaches that unmodified oligodeoxynucleotides can be converted into phosphorothioate oligodeoxynucleotides using standard phosphoramidite protocols but replacing the standard oxidation by iodine with Beucage reagent for sulfurization. Weiss teaches that using Beucage reagent results in the replacement of every oxygen group of the phosphodiester bond with a sulfur group, and that such substitutions result in an asymmetric distribution of the negative charge to predominate on the sulfur atom, resulting in "improved stability to nucleases, retention of solubility in water and stability to base-catalyzed hydrolysis" (col. 13, lines 2-14), improved biodistribution and *in vivo* stability (col. 15, lines 41-45), and activation of Rnase H, and thus are potentially useful therapeutic agents (col. 13, lines 45-47).

Weiss does not teach or suggest a method for sequencing a target nucleic acid that includes hybridizing a set of nucleic acid fragments, each containing a sequence that corresponds to a sequence of a target array, to an array of nucleic acid probes to form a target array of nucleic acids, where each probe includes a single-stranded portion including a variable region such that each member of the set hybridizes to a member of the array of probes; determining molecular weights of nucleic acids in the target array to identify hybridized probes; and based upon the hybridized probes, determining the sequence of the target nucleic acid.

ANALYSIS

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

(1) There would have been no motivation to have combined the teachings of Köster with those of Cantor and Weiss

There would have been no motivation to one of ordinary skill in the art to have combined Köster and Cantor with Weiss in the manner suggested by the Examiner. Weiss teaches methods and compositions for diagnosing and treating pathological conditions related to a dopamine receptor abnormality. The reference teaches that unmodified oligodeoxynucleotides can be converted into phosphorothioate oligodeoxynucleotides using standard phosphoramidite protocols but replacing the standard oxidation by iodine with Beucage reagent for sulfurization. Weiss teaches that using Beucage reagent results in the replacement of every oxygen group of the phosphodiester bond with a sulfur group, and that such substitutions result in an asymmetric distribution of the negative charge to predominate on the sulfur atom, resulting in "improved stability to nucleases, retention of solubility in water and stability to base-catalyzed hydrolysis" (column 13, lines 2-14), improved biodistribution and *in vivo* stability (column 15, lines 41-45), and activation of RNAse H, and thus are potentially useful therapeutic agents (column 13, lines 45-47). Since Weiss is not concerned with methods for detecting nucleic acid molecules or sequencing nucleic acids, it's teachings are unrelated to the methods of Köster or Cantor. Accordingly, those of ordinary skill in the art would not have been motivated to have combined the teachings of the references. The advantages of using Beaucage reagent articulated by Weiss are inapplicable to detection or sequencing methods.

Further, Weiss does not teach or suggest the methods of sequencing by determining molecular weights of nucleic acids in a target array to identify hybridized probes and based on the hybridized probes determining the sequence of the target nucleic acid. Weiss also does not teach an array of nucleic acid probes each of which includes a single-stranded portion and a double-stranded portion, where the single-stranded region includes a variable region, and where the probes are mass modified.

(2) Notwithstanding the lack of motivation, the combination of the teachings of Köster with the teachings of Cantor and Weiss does not result in the instantly claimed methods.

As discussed above, neither Köster nor Cantor, individually or in combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining the molecular weight of nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined. Weiss does not cure this defect. Weiss does not teach or suggest sequencing a target nucleic acid. Weiss does not teach or suggest a method that includes any of the steps of providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence in the target nucleic acid, hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, or determining molecular weights for nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid can be determined. Thus, even if Weiss teaches generation of thiol moieties using Beucage reagent, this reference fails to cure the deficiencies in the combination of the teachings of Köster and Cantor.

None of Köster, Cantor or Weiss, individually or in any combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining the molecular weight of nucleic acid fragments hybridized in the target array in order to identify hybridized probes and thereby determine the sequence of the target nucleic acid. Thus, combining the teachings of Köster, Cantor and Weiss does not result in the instantly claimed method of claim 28. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness. Applicant respectfully requests that the rejection be reconsidered and withdrawn.

THE REJECTION OF CLAIMS 71 AND 72 UNDER 35 U.S.C. §103(a)

Claims 71 and 72 are rejected under 35 U.S.C. §103(a) as being unpatentable over Köster (U.S. Patent No. 5,605,798) in view of Cantor (U.S. Patent 5,503,980) in further view of Sanghvi *et al.* (U.S. Patent No. 6,214,551) because the combination of Köster and Cantor allegedly teaches all elements of

the claims except that the selectively releasable bond is 4,4'-dimethoxytrityl or a derivative thereof, and Sanghvi *et al.* allegedly cures this defect. The Examiner contends that Sanghvi *et al.* teaches the selectively releasable bond 4,4'-dimethoxytrityl or a derivative thereof, and argues that although the reference does not teach the derivative 3 or 4 [bis-(4-methoxy-phenyl)]-methyl-benzoic acid in particular, Sanghvi *et al.* teaches equivalent compounds and derivatives used for the same purpose.

This rejection is respectfully traversed.

THE CLAIMS

Claims 71 and 72 ultimately depend from claim 1 and are directed to various embodiments thereof. Claim 71 is directed to the embodiment where each probe is attached to the solid support by a selectively releasable bond that includes 4, 4'-dimethoxytrityl or a derivative thereof. Claim 72 is directed to the embodiment where the derivative of 4, 4'-dimethoxytrityl is selected from the group consisting of 3 or 4 [bis-(4-methoxyphenyl)]-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-hydroxymethyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-chloromethyl-benzoic acid and salts thereof.

RELEVANT LAW

See related section above.

TEACHINGS OF THE CITED ART

Köster (U.S. Patent 5,605,798)

See related section above.

Cantor (U.S. Patent 5,503,980)

See related section above.

Sanghvi et al. (U.S. Patent 6,214,551)

Sanghvi *et al.* teaches compounds that mimic and/or modulate the activity of wild-type nucleic acids. The compounds taught by Sanghvi *et al.* contain a selected nucleotide sequence where the nucleotides are covalently bound through

linking groups that contain adjacent nitrogen atoms. Sanghvi *et al.* teaches the use of dimethoxytrityl groups as a blocking group during nucleoside polymerization. Sanghvi *et al.* teaches that an oligonucleotide is tethered to a solid support via its 3' hydroxyl group (col. 57, line 63 through col. 58, line 14).

Sanghvi *et al.* does not teach or suggest the use of dimethoxytrityl or a derivative thereof as a selectively releasable bond by which to attach a probe to a solid support. Sanghvi *et al.* does not teach or suggest using mass spectrometry, or using mass spectrometry for sequencing nucleic acids, or hybridizing a set of nucleic acid fragments containing a sequence that corresponds to a sequence of the target nucleic acid to an array of nucleic acid probes to form a target array of nucleic acids. Sanghvi *et al.* does not teach or suggest determining the molecular weights for nucleic acids of the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined.

ANALYSIS

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of teachings of Köster and Cantor with the teachings of Sanghvi *et al.* does not result in the instantly claimed methods.

As discussed above, neither Köster nor Cantor, individually or in combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining the molecular weight of nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined. Sanghvi *et al.* does not cure this defect. Sanghvi *et al.* does not teach or suggest using mass spectrometry, or using mass spectrometry for sequencing nucleic acids, or hybridizing a set of nucleic acid fragments containing a sequence that corresponds to a sequence of the target nucleic acid to an array of nucleic acid probes to form a target array of nucleic acids. Sanghvi *et al.* does not teach or suggest determining the molecular weights for nucleic acids of the target array to identify hybridized probes,

whereby the sequence of the target nucleic acid is determined. Hence, Sanghvi *et al.* does not teach or suggest the subject matter missing from the combination of the teachings of Köster and Cantor.

Accordingly, even if, arguendo, Sanghvi *et al.* teaches selectively releasable bonds containing 4,4'-dimethoxytrityl or a derivative thereof, which applicant contends is not taught by Sanghvi *et al.*, the combination of Köster, Cantor and Sanghvi *et al.* does not teach or suggest all the elements of the claimed methods.

None of Köster, Cantor or Sanghvi *et al.*, individually or in any combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining the molecular weight of nucleic acid fragments hybridized in the target array in order to identify hybridized probes. Thus, combining the teachings of Köster, Cantor and Sanghvi *et al.* does not result in the instantly claimed methods of claims 71 and 72. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the above amendments, consideration and allowance of the application are respectfully requested.

Respectfully submitted,
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